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ORIGINAL ARTICLE

Anti-Inflammatory and Anti-Cell Proliferative Effects of Dieckol in the Prevention and Treatment of Colon Cancer Induced by 1,2-Dimethyl Hydrazine in Experimental Animals

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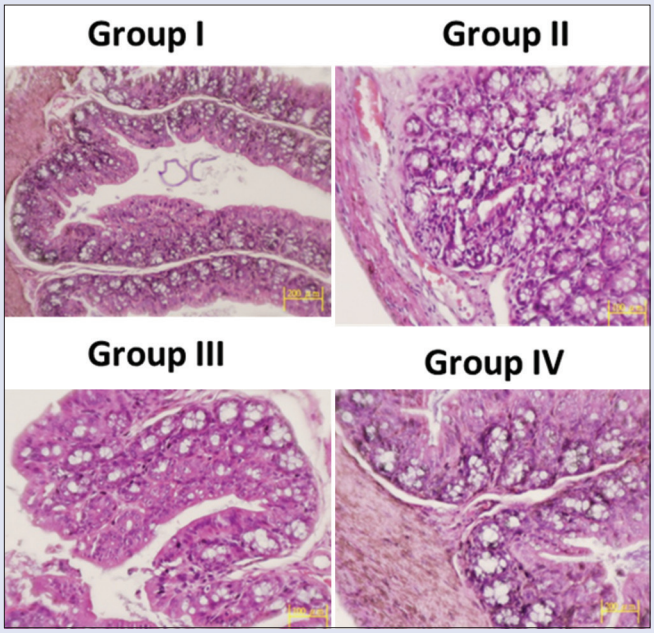
ABSTRACT

Objectives: Colon carcinogenesis is a major cause of mortality and morbidity in developing and developed countries, and its etiology is familiar to be a grouping of environmental and nutritional factors, hereditary factors, and deficiency of physical activity. In the present study, we investigated the anti-cell proliferative and anti-inflammatory effects of dieckol (DEK) on 1,2-dimethylhydrazine (DMH)-treated colon carcinogenesis in investigational rats. **Materials and Methods:** Colon carcinogenesis was induced with DMH (20 mg/kg body weight) by subcutaneous injection once weekly. We analyzed body weight, tumor incidence, tumor volume, total number of tumors, thiobarbituric acid-reactive substances (TBARS), antioxidants (glutathione peroxidase, glutathione, catalase, and superoxide dismutase), Phase II (glutathione reductase and glutathione S-transferase) and Phase I (Cyt-b_5 and CYP_{450}) biotransformation enzymes, and histopathological alterations in the control and investigational rats. Moreover, the inflammatory (interleukin [IL] IL-1β, cyclooxygenase-2, tumor necrosis factor-alpha, IL-6, and inducible nitric oxide synthase) and cell proliferative (cyclin D1 and PCNA) markers were analyzed by Western blot technique in experimental and control rats. **Results:** We noted decreased body weight, antioxidants and Phase I and II enzymes, augmented tumor incidence, tumor volume, total number of tumors, TBARS, and irregular histopathological changes in DMH-induced animals. In addition, the Western blotting analysis of colon tissues showed an upregulation of inflammatory and cell proliferative markers in DMH-treated rats. Oral supplementation of DEK inhibited the tumor formation, controlled inflammation, cell proliferation, and restoration of biochemical parameters, and it was supported by the histopathological analysis. **Conclusion:** Findings from the study suggest that DEK demonstrated anticancer, anti-inflammatory, and anti-cell proliferative effects against DMH-treated colon carcinogenesis in rats.

Key words: Cell proliferation, colon cancer, dieckol, 1,2-dimethylhydrazine, inflammation

SUMMARY

- Colon carcinogenesis is the second leading cause of cancer-related death worldwide behind cardiovascular disease. The most common types of cancer are prostate, lung, and colorectal cancers in males and breast, colorectal, and lung cancers in females
- Dieckol (DEK), a phlorotannin compound enriched in *Ecklonia cava,* is a marine brown algae with the several biological activities
- The administration with DEK reveals ameliorating anticancer effects by changing multiple processes, including Phase I and Phase II biotransformation enzymes, antioxidants, lipid peroxidation, and histopathological changes in the rat colon cancer.



Abbreviations used: DMH: 1,2-Dimethyl hydrazine; SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase; TBARS: Thiobarbituric acid-reactive substances; iNOS: Inducible nitric oxide synthase; COX-2: Cyclooxygenase-2; TNF-α: Tumor necrosis factor-alpha; NF-κB: Nuclear factor-kappa B; IL-6: Interleukin-6.

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INTRODUCTION

Colon carcinogenesis is the second leading cause of cancer-related death worldwide behind cardiovascular disease. The most common types of cancer are prostate, lung, and colorectal cancers in males and breast, colorectal, and lung cancers in females. According to results, globally, colorectal cancer claimed an estimated 880,792 lives (males 484,224; females 396,568), with 1.85 million new cases (males 1.03 million; females 823,303) estimated to be diagnosed in 2018.[1] Colon cancer is divided into three distinct steps similar to various kinds of tumorigenesis such as initiation, promotion, and progression, during which the regular colonic epithelium suffers a pathological transformation into the hyperproliferative epithelium and ultimately into invasive and metastatic carcinogenesis.[2] At present, the two most important choices for the management of colon carcinogenesis are surgery and chemotherapy, and alleviate opportunity is dependent on tumor size, position, and stage of carcinogenesis.[3] Treatment of chemotherapy may be started at all stages and is normally managed by subsequent surgery. On the other hand, in several occurrences, it also starts earlier to surgical treatment in classify to decrease the size of tumor.[4] Some studies have suggested that the number of colorectal cancer survivors has increased in the last few years because of the developments in chemotherapeutic treatments and medical procedures.[5] Colon cancer patients with chemotherapeutic approach often experience neutropenia, diarrhea, thrombocytopenia, mucositis, and palmar-plantar erythrodysesthesia. Nearly 20% of patients experience adverse toxicities and 1% of patients suffer fatal toxicity.[6] Hence, there is an urgent need to develop novel herb-based remedial agents with null or fewer side effects.

Malignant transformation of a cancer cell is differentiated by numerous key hallmarks that indirectly or directly show the way to diminish cell death, uncontrolled cell proliferation, provoked angiogenesis, and metastatic prospective.[7] However, recent evidence has demonstrated that inflammation is a fine predictable feature of carcinogenesis that was interestingly both an effect and cause of malignant conversion.[8] The genetic actions resultant malignant conversion also begin the appearance of inflammation associated mechanisms, which directs to the tumor growth of inflammatory milieu.[9] Oxidative stress is illustrious of triggering different types of transcription mediators such as nuclear factor-kappa B (NF-κB), tumor necrosis factor-alpha (TNF-α), and AP-1, leading to conversion of normal cells into tumor cells.[10]

Epidemiological data occur strongest correlate with nutritional ingestion of vegetables, foods and medicinal herbs decreased the possibility of cancer in the investigation animals and human beings.[11] Especially, marine bioactive polyphenolic agents that particularly disturb molecular and cellular pathways and conquer inflammation in different types of carcinogenesis have gained extensive attention in modern years for new anticancer-based treatments.[12] More particularly, brown algae composed of polyphenols and polysaccharide have emerged as very promising to decrease cancer development.[13] Dieckol (DEK), a phlorotannin compound enriched in *Ecklonia cava,* is a marine brown algae identified in oceanic of Japan and Korea.[14] DEK has earlier been assessed with strong antioxidant, anti-hyperlipidemic, anti-allergic, anti-skin aging, anti-tumor, anti-neurodegenerative, anti-inflammatory, and anti-diabetic properties.[15-20] Numerous previous studies have proved the anticancer potential of DEK against liver cancer[21,22] and ovarian cancer.[23] Although DEK is suggested to possess anti-cancer properties in a range of cancers, its effects on colon cancer have not yet recognized. In this investigation, we explained the function of DEK on 1,2-dimethylhydrazine (DMH)-treated colon tumorigenesis in animals.

MATERIALS AND METHODS

Chemicals

DMH and DEK were purchased from Sigma Chemical Company. The primary antibodies for PCNA, cyclooxygenase-2 (COX-2), interleukin-6 (IL-6), cyclin D1, TNF-α, IL-1β, inducible nitric oxide synthase (iNOS), and β-actin were procured from Santa Cruz Biotechnology, California, USA. All the extra chemicals were employed for the analytical ranking.

Animal model and cancer induction

Adult male Wistar animals (150–170 g) were acquired. All animals were sustained in the regular laboratory atmospheres of temperature (25°C ± 2°C), average humidity, and 12 h light/dark series. The animals were fed with regular animal pellet diet and water *ad libitum*. This research was approved by the institutional animal ethics committee. For the production of colon tumorigenesis, DMH was combined with 1 mM of EDTA and the pH was set to 6.5–1 mM of NaOH, for guaranteed strength of the carcinogen. After that, it was treated with subcutaneous injection at 20 mg/kg body weight once weekly, for starting 4 weeks of investigation.

Experimental design

The rats were randomly separated into four groups with six rats in every group. Group-1: the animals were used as normal; Group 2: the rats were induced colon cancer with 20 mg/kg bwt of DMH through subcutaneous injections 1 time in a week for 16 weeks; Group 3: the animals were induced DMH subsequent daily oral supplementation of DEK (20 mg/kg bwt); and Group 4: the rats were orally administered DEK (20 mg/kg bwt) alone daily for 16 weeks. During the study periods, body weight of the control and investigational animals was determined The body weight, tumor volume, volume, and incidences of gas chromatography were examined via the previously described method.[24] At the end of investigation time (16 weeks), the rats in various groups were sacrificed. Colon tissues were dissected out, weighed, and cleaned with iced saline. After that, the colon tissue was preserved in 10% of formalin and stored at – 80°C for histopathological investigations.

Biochemical analysis

The concentrations of the thiobarbituric acid-reactive substances (TBARS) in liver and colon tissues were examined via the technique of Ohkawa *et al*.[25] Catalase (CAT) enzyme activity of colon and liver tissues was measured via the process of Sinha.[26] Cyt-b_5 and CYP_{450} levels in colonic and liver tissues were evaluated via the process of Omura and Sato.[27] The ranges of glutathione (GSH) and glutathione peroxidase (GPx) in the colon and liver tissues were evaluated via the manner of Beutler and Kelly[28] and Rotruck *et al*.[29] Superoxide dismutase (SOD) activities were analyzed in colon and liver tissues using the process of Kakkar *et al*.[30] The glutathione reductase (GR) levels were measured via the approach of Carlberg and Mannervik.[31] The glutathione S-transferase (GST) was concentrated according to the technique described by Habig *et al*.[32]

Histopathological analysis

The colon tissues of both control and experimental animals were removed and preset in normal buffered formalin (5%), and then fixed in paraffin wax. Then, tissues were divided with a microtome, dehydrated through a sequence of ethyl alcohol, and then entrenched with paraffin. The segments of colon (5–6 μm

thick) were set up and subsequently stained with hematoxylin and eosin dye. Finally, the stained colon tissues were examined by a light microscope (Olympus, Tokyo, Japan) to detect the histological changes in the colon tissues.

Western blotting analysis

Colon tissues were homogenized in RIPA buffer and centrifuged at × 15,000 g for 15 min at 4°C. Afterward, the protein levels of lysates were measured by the Bradford system.[33] Sodium dodecyl-sulfate polyacrylamide gel electrophoresis was employed using 50 μg of protein from every sample as explained earlier.[34] Separated proteins were shifted to the polyvinylidene fluoride membrane and incubated with 1:1000 dilutions of PCNA, iNOS, IL-6, cyclin D1, TNF-α, COX-2, and IL-1β for overnight at 4°C. The membranes were kept (1 h) with 1:500 dilutions of secondary antibodies at RT. The protein bands were detected by ECL identification kit (Biorad, California, USA).

Statistical examination

The data were determined as mean ± standard deviation. Statistical investigation was done in the GraphPad Prism 8 software (San Diego, CA, USA) using ANOVA subsequent by Tukey's test as a *post hoc* test. The differences were measured as statistically significant at $P < 0.05$.

RESULTS

Effect of dieckol in body weight modifications

The alterations of body weight in the investigation and control rats are shown in Figure 1. Initially, no significant alterations were found in the experimentation and normal animals. The body weight notably ($P < 0.05$) reduced at the end of the investigation time of DMH-treated animals when evaluated with the normal rats. In contrast, supplemented with DEK revealed the considerable ($P < 0.05$) reduce body weight of DMH supplemented rats. DEK-only- and control-treated rats revealed no body weight alterations.

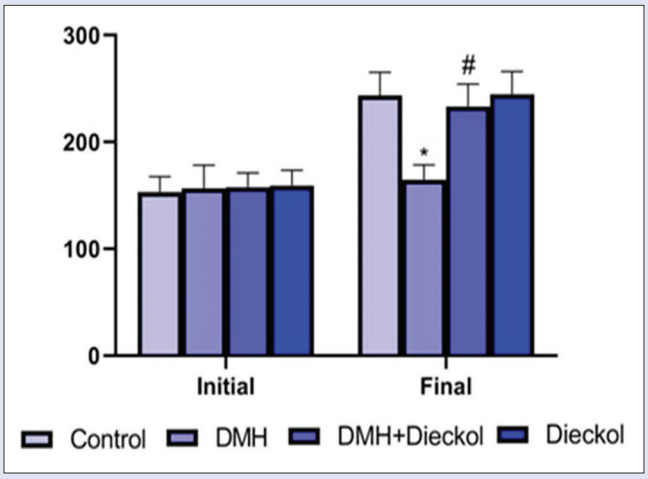

Figure 1: Initial and final body weight changes of control and experimental rats in each group. Values are given as mean ± standard deviation for groups of six rats in each. Statistical significance was determined by one-way ANOVA followed by Tukey's *post hoc* test; where $^{*}P < 0.05$ when compared with the vehicle control group, $^{\#}P < 0.05$ when compared with the 1,2-dimethylhydrazine-induced group

Effect of dieckol on tumor incidence, tumor numbers, and tumor volume

The tumor volume, incidence of tumor, and total number of tumors in experimental and normal control animals are shown in Table 1. We recognized 100% incidence of tumor, tumor volume, and increased total tumor numbers in DMH-administered animals as evaluated with control groups. Equally, oral administration of DEK on cancer-bearing animals impedes the colon cancer by did not observe any tumors.

Effect of dieckol on thiobarbituric acid-reactive substances status in liver and colon tissues

The quantity of TBARS in colon and liver tissues of normal and investigation animals is shown in Figure 2. The level of TBARS noticeably ($P < 0.05$) elevated in liver, while lowering in colon tissues of DMH induced animals when evaluated with the normal animals. Equally, DEK-treated animals were changed the levels of TBARS when evaluated with DMH induced animals. DEK-only- and control-treated rats confirmed no effects.

Table 1: Effect of dieckol on total number of tumors, tumor incidence, and tumor volume of control and experimental animals

Groups	Total number of tumors (*n*)	Tumor incidence (%)	Tumor volume (mm^3)/rat
Control	0	0	0
DMH	6	100	11.78±0.80*
DMH+DEK	6	35	4.92 ± 0.18
DEK	0	0	0

Statistical significance was determined by one-way ANOVA followed by Tukey's *post hoc* test; where $^{*}P<0.05$ when compared with the vehicle control group. Tumor volume was calculated using the formula V=4/3 (*D*1/2) (*D*2/2) (*D*3/2), where *D*1, *D*2, and *D*3 are the three diameters (mm) of the tumor; ( ) indicates total number of animals bearing tumors. Values are given as mean±SD for groups of six rats in each. DMH: Dimethylhydrazine; DEK: Dieckol; SD: Standard deviation

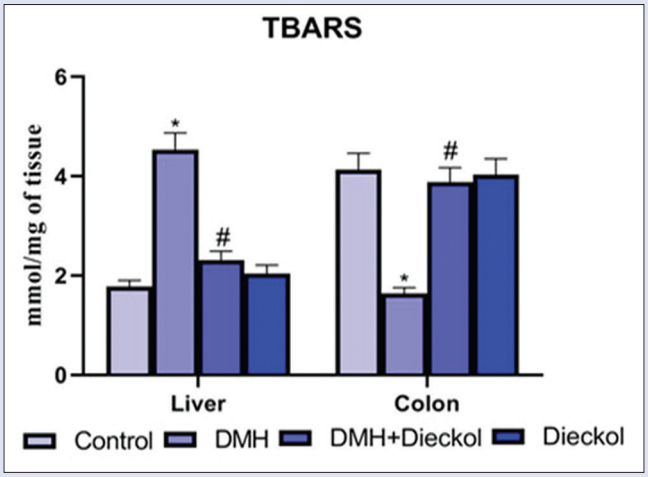

Figure 2: The level of thiobarbituric acid-reactive substances shown in the liver and colon tissues of control and experimental animals in each group. Values are given as mean ± standard deviation for groups of six rats in each. Statistical significance was determined by one-way ANOVA followed by Tukey's *post hoc* test; where $^{*}P < 0.05$ when compared with the vehicle control group, $^{\#}P < 0.05$ when compared with the 1,2-dimethyl hydrazine-induced group

Effect of dieckol on antioxidant levels in liver tissues

The level of antioxidant (SOD, CAT, GSH, and GPx) enzymes in liver tissues is shown in Figure 3. The status of antioxidants such as GSH, CAT, GPx, and SOD noticeably ($P < 0.05$) lowered in the liver of DMH-induced rats. Otherwise, DEK-administrated animals were increased these antioxidant activities when compared with DMH-induced cancer-bearing rats. DEK-only- and control-treated rats reported no effects.

Effect of dieckol on antioxidant levels in colon tissues

The level of antioxidant (SOD, CAT, GSH, and GPx) enzymes in colon tissues is shown in Figure 4. The functions of antioxidants such as GSH, CAT, GPx, and SOD markedly ($P < 0.05$) lowered in colon tissues of DMH-treated animals. On the other hand, DEK-treated animals were increased these antioxidant activities when compared with DMH induced cancer bearing rats. DEK-only- and control-treated rats exemplified no effects.

Effect of dieckol on detoxification enzyme activities in liver tissues

The level of Phase II (GR and GST) and Phase I (Cyt-b_5 and CYP_{450}) biotransformation enzymes in the liver tissue of investigational and normal animals is shown in Figure 5. The Phase I enzyme level noticeably ($P < 0.05$) elevated, and the levels of Phase II enzymes notably ($P < 0.05$) reduced in DMH-administered rats when evaluated with the normal rats. Oral administration of DEK unusually ($P < 0.05$) lowered in Phase I enzymes and evidently ($P < 0.05$) augmented in Phase II enzymes when evaluated with cancer-induced rats. DEK-only and control-treated rats showed no effects.

Effect of dieckol on detoxification enzyme activities in colon tissues

The level of Phase-I (Cyt-b_5 and CYP_{450}) and Phase-II (GR and GST) biotransformation enzymes in colon tissue of investigational and normal animals is shown in Figure 6. The concentrations of Phase-I enzymes noticeably ($P < 0.05$) augmented, at the same time as the levels of Phase-II enzymes remarkably ($P < 0.05$) diminished in DMH-treated animals when evaluated with normal animals. The administration of DEK orally noticeably ($P < 0.05$) lowered in Phase-I enzymes and markedly ($P < 0.05$) augmented in Phase-II enzymes when evaluated with cancer-induced rats. DEK-only- and control-treated rats showed no effects.

Effect of dieckol on histopathological changes of colon tissues

Photomicrographs of colon in control and investigation rats are shown in Figure 7. Histological assessment of the colon segments from control- [Figure 7a] and DEK (20 mg/kg/bwt)-only supplemented [Figure 7d] rats established the regular mucosal and submucosal layers of architecture. The DMH-only-treated animals [Figure 7b] of colon tissues explained proliferating mucosal glands with strict dysplastic alterations showing conversions to carcinogenesis. The cancer-induced rats were administered with 20 mg/kg body weight of DEK [Figure 7c] treated rats revealed glands within regular limits surrounded by lymphoid aggregates.

Effect of dieckol on western blotting protein expression of inflammatory markers in colon tissues

The status of inflammatory markers such as IL-1β, COX-2, TNF-α, iNOS, and IL-6 in colon tissues of experimental and control animals is shown in Figure 8. The expressions of TNF-α, IL-1β, COX-2, iNOS, and IL-6 markedly ($P < 0.05$) augmented in DMH-supplemented animals. Alternatively, treated with DEK induced animals were confirmed a considerable ($P < 0.05$) reduced in inflammatory marker expression as evaluated to the DMH only supplemented rats. No significant changes were found in control- and DEK-only-treated animals.

Effect of dieckol on western blotting protein expression of cell proliferative markers in colon tissues

The cell proliferative mediator expressions such as cyclin D1 and PCNA in colon tissues of experimental and control animals are revealed in Figure 9. The expressions of cyclin D1 and PCNA remarkably ($P < 0.05$) increased in DMH-treated animals. Contrarily, administration of DEK to DMH-induced animals illustrated a considerable ($P < 0.05$) reduction in cell proliferative marker expressions as found in the evaluation of

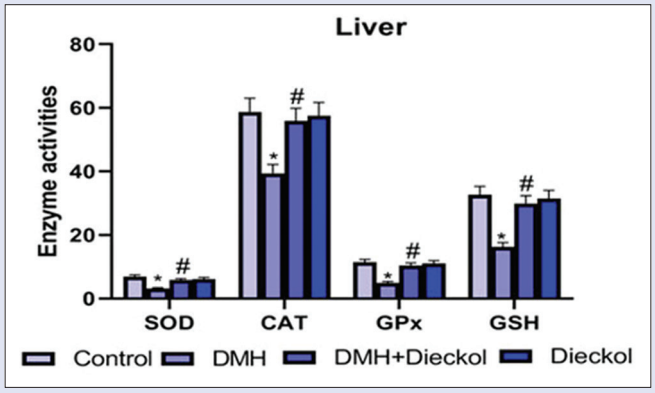

Figure 3: The level of antioxidants shown in the liver tissues of control and experimental animals in each group. Values are given as mean ± standard deviation for groups of six rats in each. Statistical significance was determined by one-way ANOVA followed by Tukey's *post hoc* test; where $^{*}P < 0.05$ when compared with the vehicle control group, $^{\#}P < 0.05$ when compared with the 1,2-dimethyl hydrazine-induced group

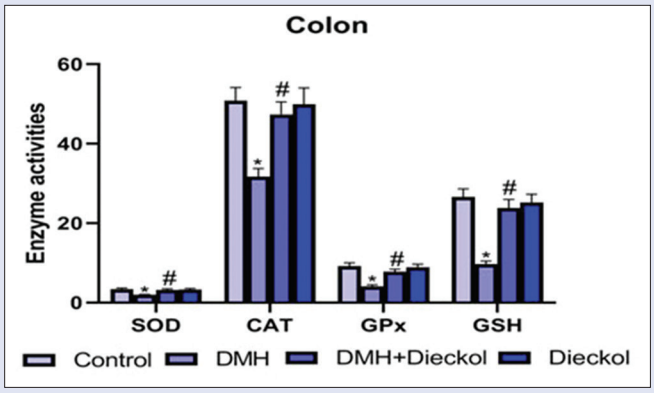

Figure 4: The level of antioxidants shown in the colon tissues of control and experimental animals in each group. Values are given as mean ± standard deviation for groups of six rats in each. Statistical significance was determined by one-way ANOVA followed by Tukey's *post hoc* test; where $^{*}P < 0.05$ when compared with the vehicle control group, $^{\#}P < 0.05$ when compared with the 1,2-dimethyl hydrazine-induced group

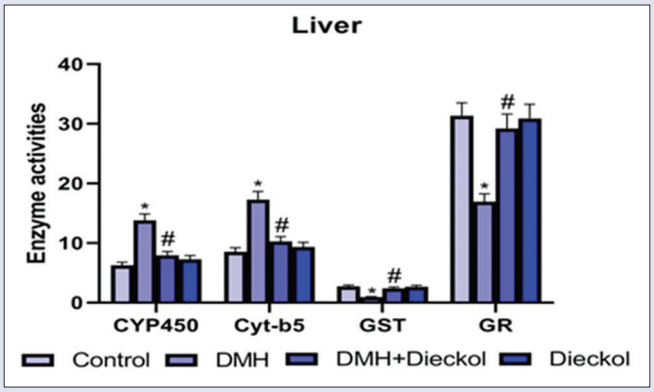

Figure 5: The level of Phase I and Phase II enzymes exposed in the liver tissues of control and experimental animals in each group. Values are given as mean ± standard deviation for groups of six rats in each. Statistical significance was determined by one-way ANOVA followed by Tukey's *post hoc* test; where $^{*}P < 0.05$ when compared with the vehicle control group, $^{\#}P < 0.05$ when compared with the 1,2-dimethyl hydrazine-induced group

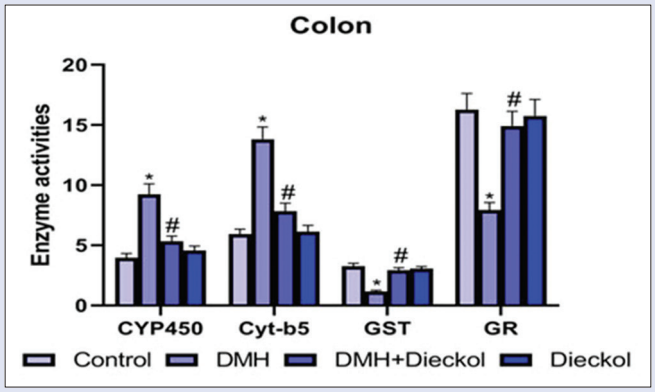

Figure 6: The level of Phase I and Phase II enzymes exposed in the colon tissues of control and experimental animals in each group. Values are given as mean ± standard deviation for groups of six rats in each. Statistical significance was determined by one-way ANOVA followed by Tukey's *post hoc* test; where $^{*}P < 0.05$ when compared with the vehicle control group, $^{\#}P < 0.05$ when compared with the 1,2-dimethyl hydrazine-induced group

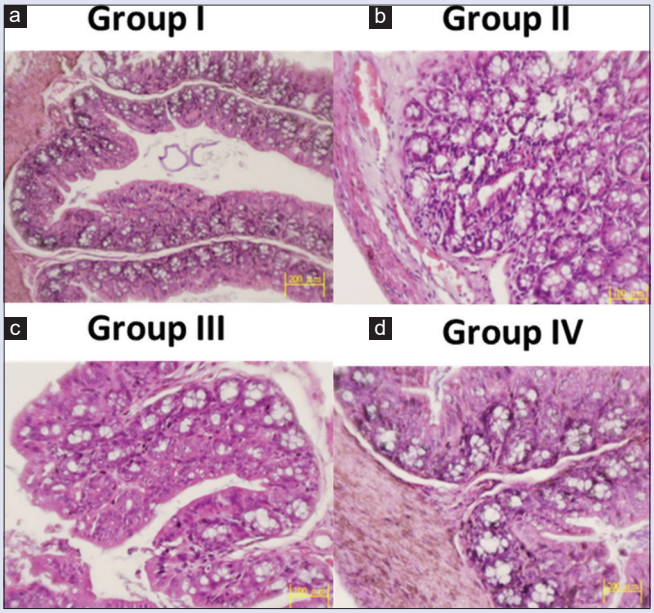

Figure 7: A cross-section of the rat colon tissues showing the histopathological changes of control and experimental rats in each group. Colon of control- (a) and dieckol- (d) treated (20 mg/kg bwt) rats demonstrating the normal architecture with mucosal and submucosal layers. Colon of a 1,2-dimethyl hydrazine-alone-treated (b) rats illustrating proliferating mucosal glands with severe dysplastic changes representing transformation to carcinoma. Colon of 1,2-dimethyl hydrazine with 20 mg/kg bwt of dieckol- (c) treated rats showing glands within normal limits

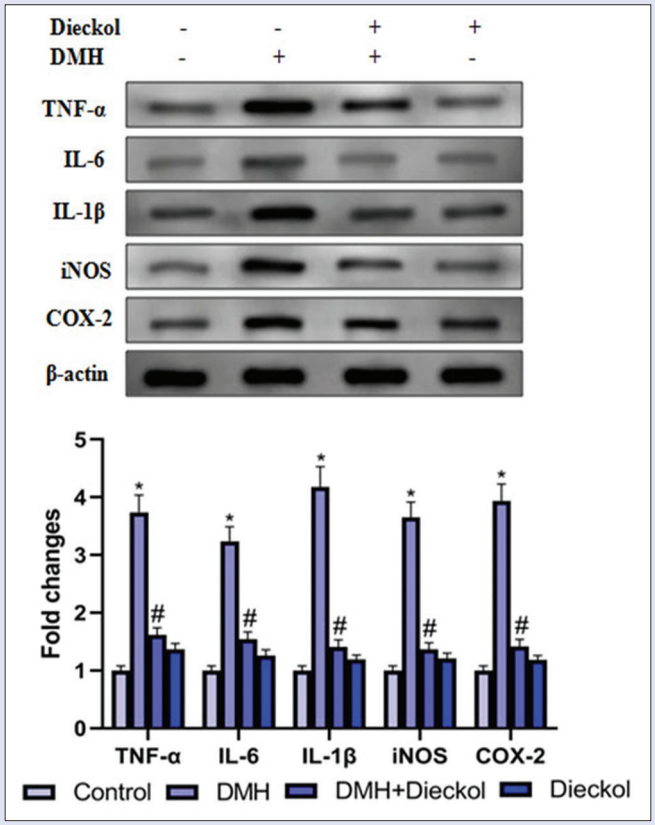

Figure 8: A representative image illustrates the effect of dieckol on inflammatory markers such as interleukin-1β, cyclooxygenase-2, tumor necrosis factor-alpha, inducible nitric oxide synthase, and interleukin-6 of experimental and control animals. The band intensities were quantified by densitometry and normalized to respective β-actin loading control. Values are given as mean ± standard deviation for groups of six rats in each. Statistical significance was measured by one-way ANOVA followed by Tukey's *post hoc* test; where $^{*}P < 0.05$ when compared with the vehicle control group, $^{\#}P < 0.05$ when compared with the 1,2-dimethyl hydrazine-induced group

DMH-alone-induced rats. No significant changes were found in the control and DEK-only-treated rats.

DISCUSSION

Colon cancer is frequently diagnosed and a pathophysiological result of the continual oxidative stress with the augmented influx of ROS.[35] The reduction in the body weight of DMH-treated cancer-induced rats may be due to the increased tumor incidence and tumor volume, accompanied

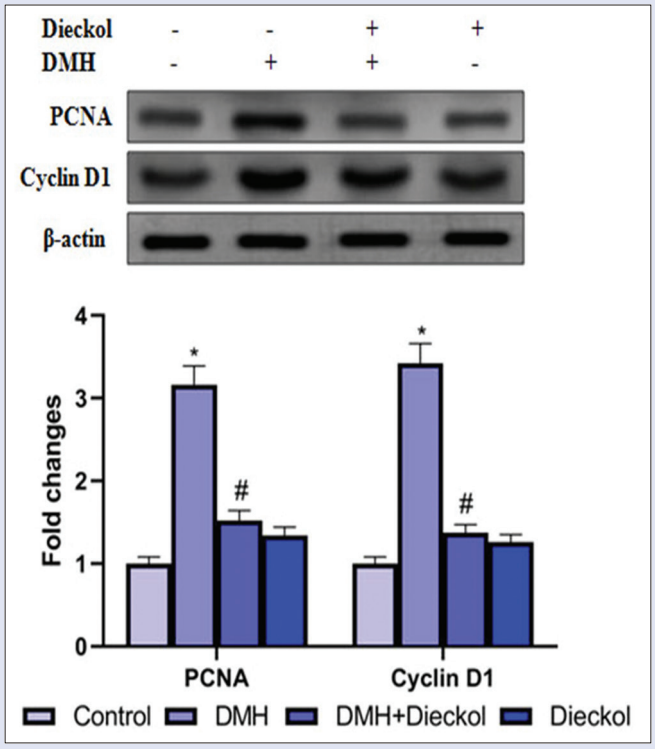

Figure 9: A representative image demonstrates the effect of dieckol on cell proliferative markers such as cyclin D1 and PCNA of experimental and control animals. The band intensities were quantified by densitometry and normalized to respective β-actin loading control. Values are given as mean ± standard deviation for groups of six rats in each. Statistical significance was measured by one-way ANOVA followed by Tukey's *post hoc* test; where $^{*}P < 0.05$ when compared with the vehicle control group, $^{\#}P < 0.05$ when compared with the 1,2-dimethyl hydrazine-induced group

by increase in polyps-driven cachexia and anorexia.[36] DEK-treated rats have revealed a significantly improved body weight regardless of the metabolic modifications treated by DMH due to their ability to restore the cellular metabolic dysfunctions. The histopathological explanation of the colon sections showed that administration with DEK considerably suppresses colon tumorigenesis by modifying the effectiveness of DMH-triggered neoplastic modifications. DMH-only-administered rats exposed proliferating mucosal glands with strict dysplastic modifications correspond to conversion of carcinogenesis.

Lipid peroxidation involves a fundamental function in tumorigenesis and may lead to generation of numerous toxic materials, such as malondialdehyde and TBARS. These toxic materials can damage cellular molecules, such as DNA, thus signifying mutagenicity and tumorigenicity.[37] Administration of DMH elevated level of lipid peroxidative products like TBARS. Animal were treated with DEK protects the cells during the suppression of lipid peroxidation as confirmation from reduced concentrations of TBARS when evaluated with rats induced by DMH. This showing anti-lipid peroxidative properties of DEK, which was possibly determined via its antioxidant potentials.[13] The antioxidants play at the first-line of protection toward oxidative stress by virtue of their ability to catalyze disproportionate reactions of their substrate free radicals that are spontaneously formed via *in vivo* cytochrome P_{450} metabolism, oxidative phosphorylation, and inflammatory processes.[38] We detected decreased levels of SOD, GPx, CAT, and GSH during DMH administration,[39] which points out the complete interruption of antioxidant-dependent mechanism of colon. The decrease in the status of these enzymes might be due to the attenuation formation or extreme consumption in trapping free radical generation. Oral supplementation of DEK-augmented levels of CAT, GPx, SOD, and GSH in the colon and liver of tumor-bearing animals, which could be due to ability to suppress lipid peroxidation, concurrently its free radical-scavenging activity. Such studies were supportive of our results that antioxidant activities are regained in DEK-administered rats and therefore protect the growth of tumorigenesis.[22] DEK could also successfully quench the free radical ROS due to their hydroxyl grouping in β-ring and electron-offering effects.[40]

The metabolic stimulation of DMH by the cytochrome P_{450} enzymes are produce active metabolites, which are most dependable for growth of tumorigenesis.[41] Ultimately, reactive products of DMH can be eliminated by Phase-II enzymes such as GST and GR. Augmented activities of cytochrome P_{450} and cyt-b_5 simultaneously with lowered activities of GST and GR were recognized in current research provides an indication of the colon cancer growth in DMH induced animals. The same results were observed earlier by DMH-treated colon cancer-bearing rats.[42,43] Our results anticipated that DEK plays a dual role via inhibiting Phase-I and improving Phase-II enzyme activities, thus supporting excretion and detoxification in DMH-treated rats. Earlier studies have informed that DEK prevents liver cancer by suppressing Phase-I and enhancing Phase-II enzymes in NDEA-induced hepatocarcinogenesis rats.[22]

Epidemiologic research have been progressively more support the impression that a powerful relationships among the inflammatory diseases and the possibility of cancer growth. Numerous important molecular goals such as IL-6, iNOS, IL-1β, TNF-α, cyclin D1, PCNA, and COX-2 have been recognized to judge the formation of inflammation and proliferation that either indirectly or directly induces carcinogenesis.[44] COX-2, an inducible prostaglandin endoperoxide synthase 2, has been associated with tumor cell proliferation and inflammation. It can be quickly stimulated by growth factors, cytokines, and tumor promoters.[45] TNF-α is a cell signaling agent, which is secreted by activated macrophages that regulate inflammation, tumor cell necrosis, and immune response.[46] IL-1β and iNOS are pro-inflammatory mediators, and these expressions have been related with augmented tumor status and aggressiveness of cancer cells.[47,48] IL-6, as a multi-functional NF-κB controlled cytokine, is a vital tumor activator in the earlier colon tumorigenesis through activating multiplication of tumor-instigating cells.[49] PCNA and Cyclin D1 are main cell proliferative mediators that can be employed for tumor growth and cell cycle development, in addition to being used as a biomarker for identification and prognosis of carcinogenesis.[17] Interestingly, several researches recognized that cytokines and proliferative markers such as IL-6, IL-1β, iNOS, PCNA, TNF-α, cyclin D1, and COX-2 are the most important factors in colon cancer progression and increased expression was observed in the colon cancer.[50-52] These findings are in line with the current understanding that augmented expression of inflammatory and proliferative markers in DMH-induced colon cancer is considered to be a chief contributor in the carcinogenesis formation. Oral supplementation of DEK reduced the levels of these inflammatory and proliferative markers due to the anti-inflammatory and anti-cell proliferative effects. Sadeeshkumar *et al.*[22] reported that DEK suppressed inflammation and proliferation in NDEA-induced hepatocarcinogenesis rats, in which is in consonance with the findings of this study.

CONCLUSION

Overall, the findings of the current research show that the administration with DEK determines ameliorating anticancer effects by changing multiple processes, including Phase I and Phase II biotransformation

enzymes, antioxidants, lipid peroxidation and histopatholgical changes. Moreover, DEK also act as an anticancer by inhibiting the inflammation and proliferation due to controlled cytokine production in DMH administered rat colon cancer. Further, molecular work is vital to identify accurate mechanism of action of DEK.

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Conflicts of interest

There are no conflicts of interest.

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